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POSTER SESSION ABSTRACTS
Session CS9 New tools for fungal biology
CS9W15

Wednesday 6th April
14:00 - 16:00

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Oligonucleotide directed mutagenesis of *Aspergilli* genomes using CRISPR-Cas9 technology

The CRISPR-Cas9 genome editing technology has recently been adapted for many species of filamentous fungi, including several *Aspergilli* species, *Trichoderma reesei*, *Neurospora crassa*, and *Pyricularia oryzae* among others. CRISPR-Cas9 induces specific DNA double strand breaks (DSBs) in the genome using a small specific RNA molecule as a guide. These breaks can then be used to destroy selected genes by relying on error-prone DNA repair by the non-homologous end-joining (NHEJ) to introduce mutations, or by increasing the efficiency of conventional gene targeting in NHEJ proficient strains. Although elimination of a gene is an efficient tool towards understanding the function of the protein encoded by this gene, it is often advantageous to introduce small specific mutations to dissect the functionality of the protein in more detail. For example, it is possible to address the importance of individual amino-acid residues in protein function by changing single codons in the gene. Similarly, by introducing subtle changes in a multi-domain protein it is possible to understand the contribution of individual domains in the overall function of this protein. In applied sciences, site directed mutagenesis can be used to optimize enzyme function by protein engineering. The classical way of introducing seamless point-mutations into the genome is by two-step pop-in pop-out gene targeting methods, which require efficient homologous recombination and a genetic marker that allows for selection as well as for counter selection. In filamentous fungi this is typically achieved by using NHEJ deficient strains and e.g. a *pyrG* marker. However construction of gene targeting substrates constitute is a tedious bottleneck towards simple high-throughput gene editing, and in some species lack of a counter-selectable marker can be a problem. Here we demonstrate a simple strategy for the generation of seamless point mutations, using short synthetic single stranded oligonucleotides and a CRISPR-Cas9 system in *Aspergillus nidulans*, and explore the parameters for efficient gene targeting, using this type of gene targeting substrate. We show that even in fungi with a well-established genetic toolbox CRISPR-Cas9 can still be a valuable addition, opening up new genetic engineering strategies.